1994 Report of the Isle of Palms Connector Study

For the Charleston Harbor Project

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I. Summary

The Isle of Palms Connector, which opened in the fall of 1993, crosses over three kilometers of productive marsh and creek habitat, including oyster fishery grounds. Previous studies indicate that runoff from highways and bridges may contain contaminants that could present hazards to this ecosystem. This study monitored the system of collector pans and spoil areas, and their effectiveness in confining potential contaminants from the highway, and slowing or stopping their entry into the ecosystem. Quarterly samples were taken of the pan sediment, the spoil areas, underneath the bridge where the pans spill over, and a control site, along with quarterly samples of the water in the pans and the water spilling over the pans. These samples were assessed using a bacterial bioluminescence assay (the Microtox), a seed growth and germination assay, and a clam growth assay. Although no statistically significant trends were found, there is a statistically insignificant tendency for the samples that are more contaminated by the highway to have a slight stimulatory effect. This effect could become more pronounced as the amount of traffic on the bridge increases.

II. Introduction

Background

Contamination of the salt marshes and tidal creeks of South Carolina's barrier islands has been an issue of increasing concern because of their great importance to man. Traditionally these regions have been productive fishing and shellfishing areas, and provide South Carolina with at least $8 million of commercial fishing revenues, and $20 million of recreational fishing revenues annually. The Carolina estuaries are either a home to or a nursery for many animals, including commercially and recreationally important species of fish in the area like croaker, drum, flounder, kingfish, menhaden, mullet and spot (Meyer 1992). The estuaries contain the base of the food web for nearly 95% of the commercially valuable fish and shellfish of the Atlantic coast (Meyer 1992). The salt marshes are important barriers against storm waters, and the loss of these marshes can lead to massive erosion (Hershner and Lake 1980). The salt marsh's ecology is tied to the ecology of endangered species like the bald eagle (*Haliaceetus leucocephalus*), and the wood stork (*Mycteria americana*), that feed off the fish in the creeks and rivers of the marsh. Also the shortnose sturgeon (*Acipenser brevirostrum*) is another endangered species in South Carolina that lives and feeds in the estuaries (Official WWF guide to the Endangered Species of North America).

The populations of the coastal cities and towns are growing rapidly. On the 148 thousand square miles of Atlantic coastal area, there were 44.5 million residents in 1960, 53.7 million in 1980, and 59.5 million in 1991 (Statistical Abstract of the United States 1993). As the population and economy of the coastal areas increase, toxicological pressures will be added to these sensitive marshes from agriculture, industrial processes and urban runoff.

One specific source of urban runoff is highway runoff. Although the runoff from highway right-of-way is generally only a small fraction of the overall stormwater pollutant loadings to surface waters, enough evidence of the detrimental effects of road runoff exists to make this an area of concern. Some of the first work that suggested the possibility of harmful effects from road runoff was in the early seventies (McGriff 1972, Wallace 1971). Since then numerous large scale studies have been conducted to try to assess the full implications of this problem (Shubinski 1975, Gupta 1981, USEPA 1983).

Literature Review

There are several ways that highway runoff can affect aquatic systems. Driscoll (1976) is one author that has shown that there is a significant impact because of the depletion of dissolved oxygen in water bodies that receive highway runoff (Driscoll 1976, Lager 1977, Gupta 1981, USEPA 1983). Dissolved oxygen depletion seems to be more dependent on the type of system studied, because the data do not seem to support generalizations to all systems. A Federal Highway Administration (FHWA) study in 1981 showed that there were notable amounts of nitrogen and phosphorus in highway runoff (Gupta 1981). The total nutrient amounts were small compared with the input from the watershed, but it is possible that they can accelerate the process of eutrophication in some areas (Kluesener 1974, Cowen, 1976a, Cowen, 1976b, Best 1978, Browman 1979). Blaurock found that biological molecules like cystine, serum albumin and glycerinaldehde phosphate dehydrogenase were destroyed by oxidatively reactive particles from gasoline exhaust (Blaurock *et al.* 1992). This effect has never been tested in the field, but it still proposes an interesting method of action for impact of highway runoff on aquatic systems. The effect of road deicing salts like sodium, calcium and chloride in freshwater systems is the most common concern of road runoff studies (Judd 1970, Cherkauer and Ostenso 1976, Hoffman *et al.* 1981). Table 1 is a list of common highway contaminants that are not from road deicing and their sources. These are the metals and hydrocarbons that might affect the biota near the Isle of Palms (IOP) connector.

Metal pollution is a cause for concern because of the persistence of metals and their potential for bioaccumulation (Guthrie *et al.* 1979). Most metals found in highway runoff are associated with the particulate phase of the runoff (Gupta 1981). It is rare to find elevated metal concentration in the water column from highway runoff because the particulate phase will settle out relatively quickly and contaminate the local sediments (FHWA 1985). This particulate phase moves from its source on the highway into the environment when the rain washes soot particles, oils and other contaminants into the estuary. Also the wind suspends the particles into the air, and then they fall into the water as the wind dies (Spies *et al*. 1987). Platinum from catalytic converters is one source of sediment contamination (Wei, Chen and Morrison). Other metallic toxicants that are found in highway runoff are cadmium, copper, chromium, iron, lead, nickel and zinc (Gupta 1981, Gjessing *et al*. 1984, Yousef *et al*. 1984, FHWA 1985.)

There are many types of polycyclic aromatic hydrocarbons (PAHs) that are associated with sediments contaminated by highway runoff that have been proven to be toxic (Table 2). One source of PAHs is exhaust particles from gasoline combustion (Volkman 1992). As cars pass over roads, materials from their tires slowly accumulate on the street surface. Lubricating oils and other fluids drip from cars and gather on the road surface. Volkman (1992) has implicated these oils as a major source of hydrocarbons in estuarine sediments in an . Some researchers have found anti-oxidants used in curing car tires, like benzothiazoles, can be washed into estuarine sediments (Spies *et al.* 1987). Runoff from the M1 roadway in England has been shown to have toxic effects because of

Table 1. Common road runoff contaminants and their probable sources. (Kobriger and Geinopolis, 1984)

Constituent	Possible sources
Particulates	Pavement wear, atmospheric deposition from vehicle exhaust and from general air pollution
Lead	Leaded gasoline exhaust, tire wear (lead oxide filler material), lubricating oil and grease drips, bearing wear
Zinc	Tire wear, motor oil (stabilizing additive), grease
Iron	Rust from automobiles, steel highway structures, moving engine part wear
Copper	Metal plating in automobiles, bearing and brushing wear, moving engine part wear, brake lining wear
Cadmium	Tire wear
Chromium	Metal plating in automobiles, moving engine part wear, brake lining wear
Nickel	Diesel fuel and gasoline exhaust, lubricating oil, metal plating in automobiles, brushing wear, brake lining wear, asphalt paving
Manganese	Moving engine parts
Bromide	Gasoline exhaust
Hydrocarbons	Spills or leaks from automobile fuels, antifreeze or hydraulic fluids, asphalt surface leachate
Benzothiazoles	Tires (Spies 1987)
Hopane	Automotive lubricating oil (Volkman 1992)
Hopene	Automotive lubricating oil (Volkman 1992)
Sterane	Automotive lubricating oil (Volkman 1992)

polycyclic aromatic hydrocarbons like pyrene and fluoranthene (Alastair Boxall, personal communication).

Table 2. Hydrocarbons that have been found in sediments contaminated by roadway runoff.

Hydrocarbon	**Author**
1,2-Benzanthracene	Shubinski 1975
3,4-Benzofluoranthene	Shubinski 1975
11,12-Benzofluoranthene	Shubinski 1975
Anthracene	Shubinski 1975
	Gjessing 1984
Benzo (e) pyrene	Gjessing 1984
Benzofluoroanthenes	Gjessing 1984
Benzothiazoles	Spies 1987
Bis(2-ethlyhexyl)phthalate	Shubinski 1975
Butyl-benzyl-phthalate	Shubinski 1975
Crysene	Shubinski 1975
	Gjessing 1984
Di-n-butyl-phthalate	Shubinski 1975
Fluoroanthene	Shubinski 1975
	Gjessing 1984
	Boxall
Hopane (sometimes methylated)	Volkman 1992
Hopene (sometimes methylated)	Volkman 1992
Naphthalene	Shubinski 1975
Phenanthrene	Shubinski 1975
	Gjessing 1984
Phenols	Shubinski 1975
Pyrene	Shubinski 1975
	Gjessing 1984
	Boxall
Sterane	Volkman 1992
Triphenylene	Gjessing 1984

Rationale

Despite the previous work done on this environmental problem, the effects of roadway runoff are not well understood in marine or estuarine systems. For this reason, the Isle of Palms connector was an issue of concern for many people even before it was built. The proposed bridge would connect the city of Mount Pleasant with the Isle of Palms. Fishermen and land owners were concerned that the bridge would affect their lives, and put up considerable legal resistance. After hurricane Hugo and the destruction of the Ben Sawyer bridge, most realized the need for an alternate evacuation route with a fixed span bridge.

The bridge was constructed one segment at a time, with each segment forming the base of operations for building the next. A giant crane traveled back and forth on the half-built bridge brought the bridge segments out to the construction area at the end of the bridge. This construction method did not require a road plowed through the marsh that would disturb the area. Unlike other bridges, the pilings were driven into the sediments below the bridge in a way that would not disturb the marsh except where the pilings pushed the sediments out of the way. In addition the Isle of Palms Connector was built with a truly unique system of environmental safeguards.

Metal concentrations in bridge runoff from conventional bridges are influenced by exactly how the stormwater gets into the receiving water. The 1985 FHWA study showed the highest metal concentrations in lake sediments from highway runoff (two to eight times control levels) were under a bridge scupper drain. The study theorized that the concentration of contaminants was so much higher here than in some of the other sites because the scupper drains do not allow for any settling out of the particulate phase of the runoff (FHWA 1985). A study of metal contamination in sediments under bridges over lake Ivanhoe, Florida (summarized in Table 3) showed that there were highly significant differences in the concentrations of seven metals in sediments under "drain and gutter" type bridges and bridges with scuppers (Table 3, Yousef 1982). The bridges in the Florida study had an average daily traffic (ADT) of 20,000 to 40,000 vehicles per day (VPD) (Yousef 1982). The Isle of Palms Connector's year 2000 predictions are only 8,500 VPD, and so the amount of metal contamination will not be nearly as high (SC DOT 1979). Yousef's study only suggests that there are better management practices than just letting the runoff drain into the watershed through scupper drains.

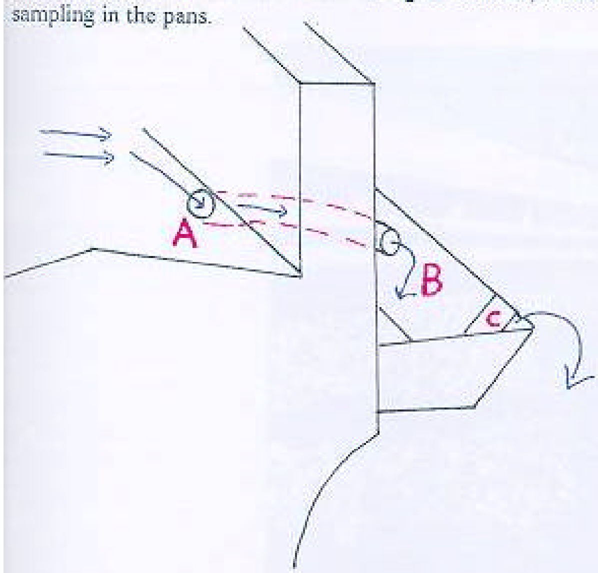
Table 3. Heavy metal concentrations of lake sediments. (Yousef, 1982)

Element	# of Observations[a]	Scuppers[b]	Drain and Gutter[b]	Probability
Zinc	8/7	96.9	42.0	99.60
Lead	8/7	423.0	132.0	99.99
Chromium	8/7	23.9	11.0	97.07
Nickel	8/7	7.2	2.8	99.60
Copper	8/7	80.1	29.2	98.71
Iron	8/7	1689.0	643.0	99.85

[a] Eight samples from the bridge with scuppers, seven sample from the bridge without scuppers
[b] Mean value in micrograms/gram dry weight of sediment

On the Isle of Palms Connector, the two-kilometer causeway has pans beneath it to collect the rain-water that runs off the bridge. When the rain hits the bridge, it runs into gutters on the side of the road. Pipes carry the runoff from the gutters to pans placed slightly under the bridge (Figure 1). Theoretically, the water will stay in the pans and evaporate after the rain stops

Figure 1.
The IOP connector collects runoff on the causeway by piping it into pans that hang slightly beneath the bridge. The roadway is cambered so that the rainfall goes into gutters on the side of the road (a), that feed the runoff into pipes that lead to the pans (b). The pans will overflow after 0.05 inches of rain fall, but metal baffles (c) will theoretically prevent most polar compounds from going into the marsh. The pans are only over the causeway, and are interspersed with light stanchions (d). The stanchions have no lights on them, so they make an ideal platform for sampling in the pans.



and the pollutants will be left in the pans as a solid residue. Regardless, the catch-pan system should allow for the particulates to settle out, so that the most toxic phase of highway runoff is contained.

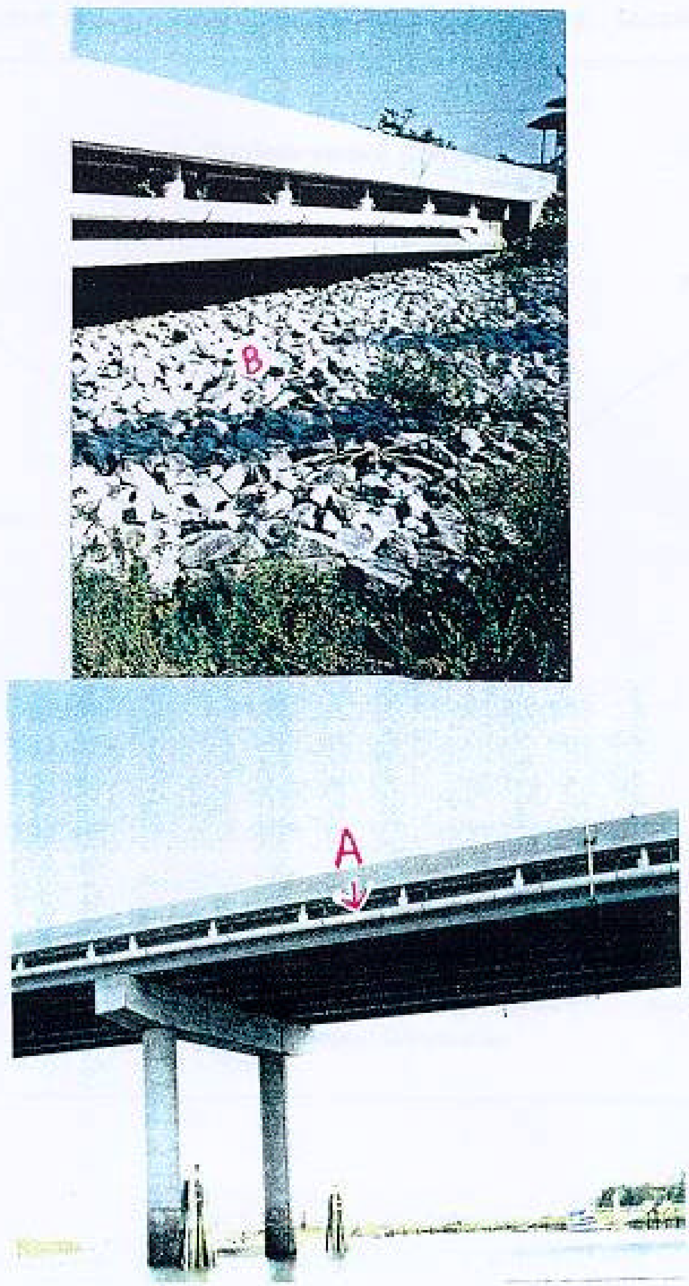
The one-kilometer span over the intra-coastal waterway has pipes that re-direct the run-off into two spoil areas (Figure 2). The spoils on the Isle of Palms side are gravel coated, marsh-like areas, where the particulates can settle out and natural processes can detoxify the run-off. Bacteria, fungi and yeasts will metabolically degrade some PAHs into less toxic byproducts in the spoil areas (Bauer and Capone 1985, Da Silvia Pinto *et al* 1979, Guerin and Jones 1989, MacGillivray and Shiaris 1993, Sutherland 1992). The spoils on the Mount Pleasant side are stone lined pools that will contain the runoff as it degrades. Some contaminants will degrade with time, or they will photodegrade. Particulate matter will settle out into the mud and will be covered up so that it is no longer biologically available. Although these sound like good ideas, this design strategy is untested, and the Isle of Palms Connector is absolutely unique because of its architecture.

Every thirty days the South Carolina Department of Transportation (SCDOT) theoretically vacuums the water and material from the bottom of the pans into a truck with a holding tank. The truck dumps its tank into the gutters of the bridge over the intracoastal waterway, and the material will flow down those gutters into the spoil areas.

One problem with the catch-pan system is that the pans are only designed to accommodate 0.5" of rain over the area of the bridge. It often rains more than 0.5" in this sub-tropical climate, and so the pans should spill over. The second problem is that the pans ice over in the winter, so that any additional rain that occurs before the ice melts will spill over. The layer of ice on top of the pans can get up to two and one half inches thick. Another difficulty with the bridge is that there have been complications with the pan-vacuuming schedule and they are not

Figure 2.

Over the intracoastal waterway there are no pans. The runoff still flows from the cambered roadway to gutters, but the pipes feed into larger pipes (a). The larger pipes are on the both the north and south sides of the bridge. On the Mount Pleasant side, the pipes feed into large gravel lined pools. On the Isle of Palms side, the pipes feed into a large gravel bed that blends into the marsh (b). These areas allow particulate matter to settle out, and allow a large surface area for natural processes to start breaking down, covering up or sequestering anthropogenic contaminants.



vacuumed out every month. Additionally the pipes, pans, gutters and the rest of the bridge architecture leak and directly spill the roadway runoff into the marsh.

III. Materials and Methods

This report is on the first year of an extended study that will monitor the effectiveness of the system of collector pans and spoil areas in confining potential contaminants, and slowing or stopping their entry into the ecosystem. These data and conclusions set an important first year baseline and elucidate worthwhile avenues for future research.

Sampling

There were two main types of samples: freshwater/runoff samples and estuarine sediment samples. They were collected quarterly to assess what trends were apparent in this system due to possible contamination from the bridge.

Pan Water: This was a sample of water that had accumulated in the pans but was not spilling over. This was collected by dipping the sample jar into the pan.

Pan Runoff: Water that was taken from the stream of water that is spilling over into the marsh. This was collected by putting a scoop into the stream of water that is spilling over the pans during a rainstorm.

Pan Sediment: This is a sample of the particulates, debris and trash that accumulated in the pans. It was collected by scraping the bottom of the pan with a scoop and then transferred to a sample jar.

Swinton Creek: Swinton Creek sediments should be typical of sediments under the causeway. They were collected by a petite ponar grab from the bridge, and then transferred to a sample jar.

North Spoil: The sediments from the north side of the spoil area on Isle of Palms were collected by scraping up the first few centimeters of sediment with a sample jar.

South Spoil: The sediments from the south side of the spoil area on Isle of Palms were collected by scraping up the first few centimeters of sediment with a sample jar. Both the North and South Spoil areas should give indications of a "worst case" scenario because they receive so much bridge runoff. These sites can be reached on foot from the Isle of Palms side of the bridge.

Control Sediment: This sediment has been collected from a wooden walkway over Dewees inlet, behind #12 and #13 Seagrass lane in Wild Dunes. Although this sediment is not necessarily classified as "clean" or "uncontaminated" it comes from an area where the sediment qualities should not change due to urbanization over the next few years. It could be a useful reference point for the continuation of this study.

Sediments were chosen as the best indicator to evaluate the effects of highway runoff in this system for several reasons. First, the literature shows that the sediments are the primary sink for the contaminants in highway runoff because the particulate phase of the runoff settles out rather rapidly (FHWA 1985). Secondly, the water in the IOP watershed is changed out completely with each high tide so water quality measurements would be of questionable worth (SC DOT 1979). Third, the key producers and the first consumers in the salt marsh are benthic or sediment dependent. Significant impacts to sediment quality could alter this ecosystem from the bottom up (Paine 1980).

Only the top layers were collected because 1) the bridge is so new, it is unlikely that deeper sediments are contaminated by bridge runoff, 2) in general, bridge contaminants tend to be in the upper sediment layers (Gjessing 1984) and 3) so that the redox potentials of the sediments are all similar.

The samples were stored in the acid and solvent cleaned glass jars that they were collected in. Some samples could not be collected with the clean glass jars. The Swinton Creek sample was collected with a ponar, and the ponar was properly cleaned before each sampling run to prevent cross-contamination. The Pan Runoff and Pan Sediment samples had to be collected remotely so a plastic scoop was used. A plastic scoop will not react with any metals present in the sample. The samples were stored in coolers with ice packs in the field. In the lab they were stored in a refrigerator at four degrees Celsius to preserve the sediments (USEPA

1991). Most of the assays were performed within two to four weeks after collection of the sediments so that there were not major changes in the toxicity of the sediments due to degradation of contaminants or due to the microbial community in the sediment.

Bioassays

Simple mortality assays were not utilized in this study for several reasons. The sediments were not expected to be very toxic so that measuring lethality would not have been useful. The variance in the mortality tests can be very high, and the number of possible replicates are low. The most likely scenario in mortality tests would be to get very little mortality, accept the null hypothesis and then the power would be very low (the chance that the test supported the conclusion of a false negative would be very high.) The oyster larvae and grass shrimp assays that were originally considered for this study are not really the most appropriate methods to assess the effects of the Isle of Palms connector. They are both tests that apply to *marine/estuarine water samples only*. The only way that sediment samples could be used with these assays would be to centrifuge the pore water out of the sediment. This would not test the effects of the toxins that could be bound within the organic particles in the sediment. The samples from the bridge could not be used with these assays at all. Therefore an alternate assay that measures the growth of *Mercenaria mercenaria*, the Atlantic Littleneck Clam, was done instead.

Three factors that affect the possible consequences of having contaminants in the environment are the level of the food chain affected by the toxins, the importance of the toxin-sensitive species to the community and the feeding mechanisms of animals in the community.

Following this rationale, the bioassay species chosen should be important to the local ecology, they should represent various levels of the food chain and they should have different feeding mechanisms. The species that were chosen for this study are *Photobacterium phosphoreum* (*Vibrio fischeri*), *Latuca sativa* and *Mercenaria mercenaria*. These organisms represent three different kingdoms, three different trophic levels and three very different forms of nutrition. The bioassays with these species represent differing tradeoffs between high levels of ecological relevance and laboratory control (Figure 3). Laboratory control encompasses speed, repeatability, accuracy and precision. The Microtox has the highest laboratory control, and the clam assay has the highest ecological relevance. (Persoone 1988, Persoone, personal communication, Hansen, personal communication.)

Microtox

In this bioassay, bioluminescent marine bacteria (species *Photobacterium phosphoreum* also known as *Vibrio fischeri*) are incubated with a sample. After the incubation period, the light output of each sample is measured in a M500 photometer-incubator (Microbics corporation). Pollutants in the sample will interfere with the oxidative respiration of the organism, and as the amount of ATP available to the system declines, the energy available to the biochemical pathway of luminescence declines. The computer (MTX7 software, Microbics corporation) will calculate an EC_{50}, or the amount of sample that it would take to reduce light output by 50%.

In the solid phase test 12 one-to-two dilutions of a 10% sediment solution are run with 3 controls. This test generates an EC_{50} in percent sediment. There is a separate protocol for liquid samples where the loss of light output due to increasing concentration of the sample is

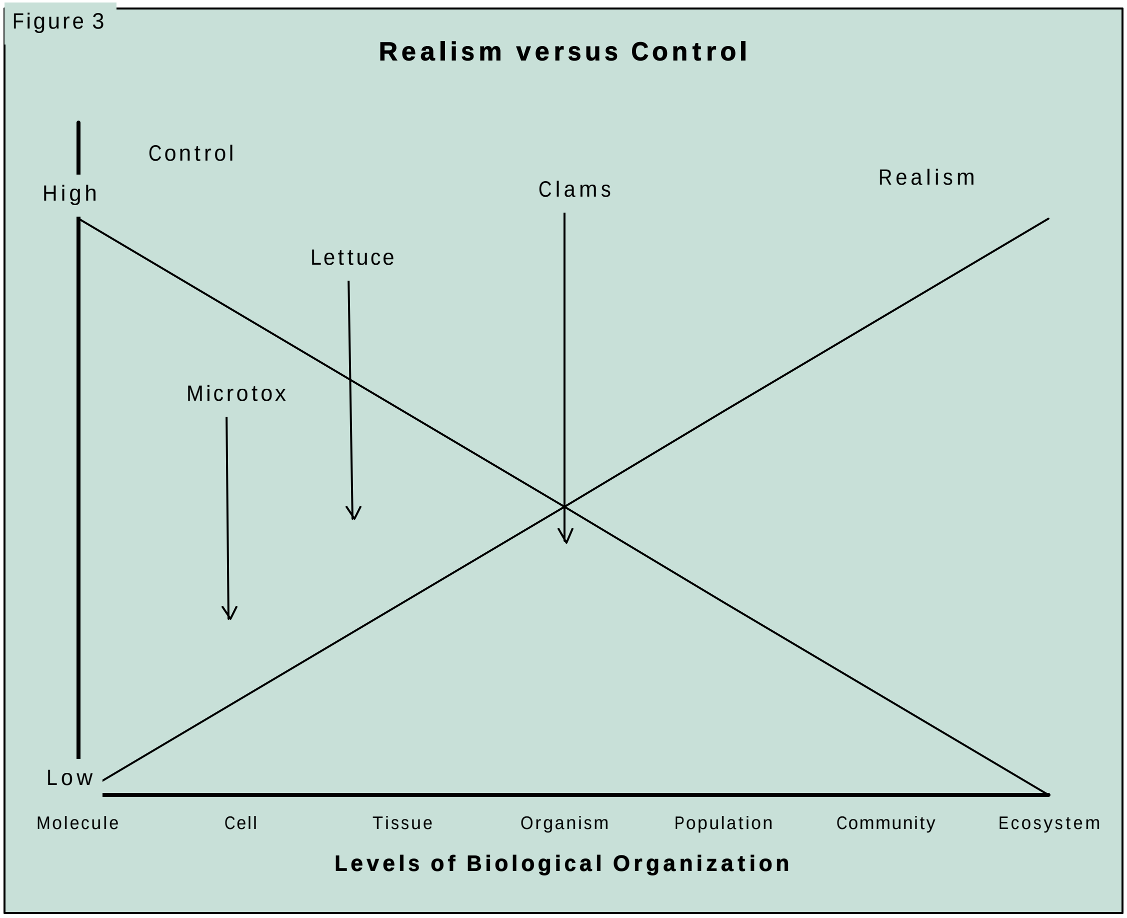


Figure 3.
Environmental realism versus control in bioassays. (Adapted from Persoone 1988, personal communication, and Hansen, personal communication). In most bioassays there is a tradeoff between experimental control of confounding factors and environmental realism. The diagonal line marked "Control" indicates a gradient of high experimental control to low amounts of experimental control. The line marked " Realism" indicates a gradient of low environmental relevance and realism to high amounts of environmental relevance and realism. The X axis shows the amount of control and realism that might be typical at these levels of biological organization.

measured at 5 minutes and 15 minutes. This assay is osmotically adjusted so that the fresh water will not adversely affect the bacteria. The liquid test is run with one control and four dilutions of the sample, and generates an EC_{50} in concentration of liquid sample.Each of these tests were run in triplicate on every sample.

The Microtox assay is often criticized when used in a system like this because it is not a local species and it is not a benthic species, and is therefore of questionable ecological value to this system. The advantages of the Microtox are that it is not time-consuming, it is very precise and it is very repeatable. It is a standardized assay that will allow other researchers to compare the toxicity of these sediments at this time to reference sites or the same sites many years later so that there will be a basis for future research that is unaffected by factors that affect most organismal bioassays. In most organismal bioassays the results can be affected by collection site, method or season for field collected animals or by culture conditions or organismal selection for lab raised organisms. This reduces the repeatability of most bioassays, but not the Microtox.

Lettuce

Wang (1985, 1986, 1987, 1991, 1992), as well as many other authors (Beckett and Davis 1978, Wollan *et al*. 1978, Wang and Williams 1988, USEPA 1982, FDA 1987, OECD 1984) have pointed out that plant seeds are a good system for toxicity testing of heavy metals, complex effluents, pesticides and hydrocarbons for several reasons. 1) Most seeds are commercially available in large quantities at low cost. 2) They can withstand long periods of desiccation/dormancy so they are available in all seasons. 3) They are sensitive to a wide array of chemical influences (Beckett and Davis 1978, Behira and Misra 1982, Cheung *et al*. 1989, Fletcher *et al*. 1985, Mrozek 1980, 1982, Ratsch and Johndro 1986, Wadell and Kraus 1990, Walsh *et al*. 1991, Wang 1985, 1986, 1987, 1991, 1992.) 4) Seeds provide information about sensitive, non-lethal endpoints like growth. 5) They can be used to test solids, liquids, sediments, turbid and colored samples and 6) most plant seed bioassays do not require large amounts of equipment or high levels of technical expertise to perform successfully.

Plant seeds can be especially sensitive because one of the first steps of germination is imbibition (Mayer and Poljakoff-Mayber 1982). In this stage the seed absorbs water, and can be especially vulnerable to contaminants in that water. Also, the seedlings have higher rates of metabolism, nutrient transport and cell division. These delicate biochemical pathways provide many sites for inhibition of these processes by anthropogenic contaminants (Wang 1991).

Although lettuce is not a species that is important to this ecosystem, it is a commonly used organism in toxicity testing. Testing some kind of plant is important for any suite of bioassays because there is no better way to determine the possible impacts on the plants in the community. Lettuce is very easy to work with and commonly available all year. In this assay 10 grams of sediment are mixed with ten milliliters of deionized water in 10 centimeter petri dishes. Then filter paper and ten seeds were added to each petri-dish. The dishes were placed in a lighted incubator set to 27 degrees Celsius for 96 hours. At the end of 96 hours, the growth of the lettuce seeds in millimeters and the percentage of seeds that germinate are recorded.

Clams

Mercenaria mercenaria, or the Atlantic littleneck clam, is a commercially important infaunal bivalve common to this area. This species is easy to acquire and work with. These animals are filter feeders, but the water that they filter comes from the very top layer of the sediment, so that they have an opportunity to be exposed to the toxins in the sediment. Clams and mussels have been shown to biomagnify heavy metals more than crabs, so that if there is an exposure, they are very likely to show some effects (Guthrie 1979). Also, extensive studies with other mussels have shown that their growth (or lack of it) is a valid environmental indicator (Salazar, 1993).

The clams were acquired from Atlantic Littleneck Clam Farms. Previous research has shown that the smallest clams (500 to 750 micrometers) display the most sensitive results in growth assays with contaminated sediments (Amy Ringwood, personal communication). One-hundred milliliters of sediments that were press sieved through a 425 micrometer screen were added to 600 milliliter beakers. Then 400 milliliters of 1 micrometer filtered seawater of 26 parts per thousand salinity was added to each beaker. The beakers were covered with parafilm to prevent evaporation, and the sediment was allowed to settle for an hour. A pipet connected to an aquarium pump was added to each beaker for aeration. The pipet was about halfway between the sediment and the surface so that the water was circulated, but the sediment was not disturbed. The beakers sat overnight so that the water was well aerated and the sediment had fully settled out.

The clams were randomly divided out into the test beakers, with 50 set aside for initial weights. Approximately 50 clams went into each beaker, the beakers were re-covered with parafilm and left undisturbed for seven days. The air sources were checked daily to insure that they were working and in the right position above the sediment. The group set aside for time zero weight was rinsed with fresh water and then dried at 70 degrees Celsius overnight to get an initial dry weight. At the end of seven days the clams were sieved out of the sediments with a 425 micrometer sieve, and then placed in beakers filled with clean 1 micrometer filtered seawater for an hour so that they would expel the sediments in their mantle cavities. The clams were rinsed in fresh water to remove the salt crystals, and then dried at 70 degrees Celsius overnight to get the dry weights.

IV. Results

Data Analysis

This study involve testing null hypotheses that all take the general form:

Null hypothesis: The means of all of the samples are equal.

Alternate hypothesis: The means of all of the samples are not equal..

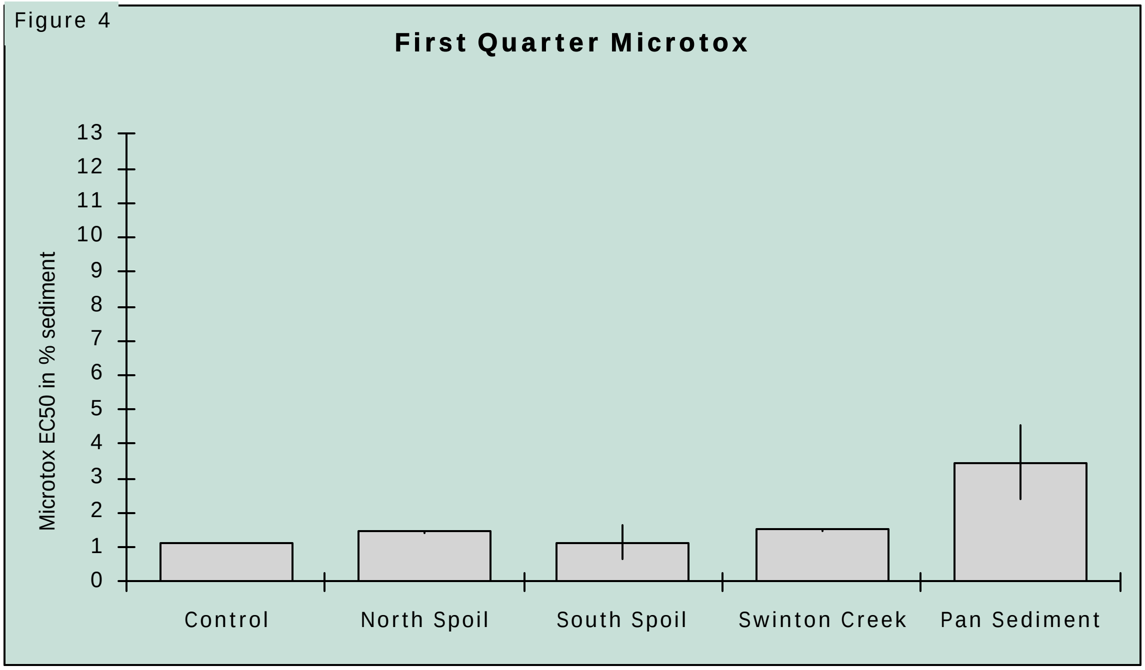
Because there were stimulatory effects, a one tailed analysis was not suitable. Before any of the data was subjected to parametric analysis, the data were tested for equal variances, normal distribution, and conformity to the central limit theorem to make sure that parametric tests e appropriate. In every case the variances were unequal (the data was heteroscedastic) so parametric tests were not appropriate.

A Kruskal-Wallis test (essentially a nonparametric ANOVA) was performed for each bioassay for each quarter, and for each bioassay for the four quarterly averages. Then nonparametric multiple comparisons were performed whenever the null hypothesis was rejected to ascertain which sites had statistically significant different means. Alpha was set at 0.05, which means that there is only a 5% chance that any of the positive results (when the null hypothesis was rejected) are false positive. In the yearly tests, where the null hypothesis of no difference was accepted, a power analysis was done to assess beta, or the chance of a false negative.

Microtox

The Microtox quarterly and yearly EC_{50}s in percent sediment results are expressed in figures 4 through 9 and table 4. The lower the Microtox EC_{50} the more toxic the sample is. The variance prohibited use of parametric statistics, so the points that are marked with asterisks as statistically different were generated with a Kruskal-Wallis test and nonparametic multiple comparisons. The power of the yearly comparison is less than 30% so there is a greater than 70% chance that this is a false negative.

The liquid samples of pan water and pan runoff were not toxic enough in any sample to calculate a Microtox EC_{50} so no statistical analysis could be performed on this data. In most cases, the sample actually *increased* light output very slightly.



Figures 4-9
These are graphs of the Microtox data from 1994. Each bar is an average EC_{50} in percent sediment of three replicates. The bars show plus and minus one standard error. The asterisks indicate statistically significant differences from a Kruskal-Wallis nonparametric ANOVA and nonparametric multiple comparisons. Alpha is 0.05.

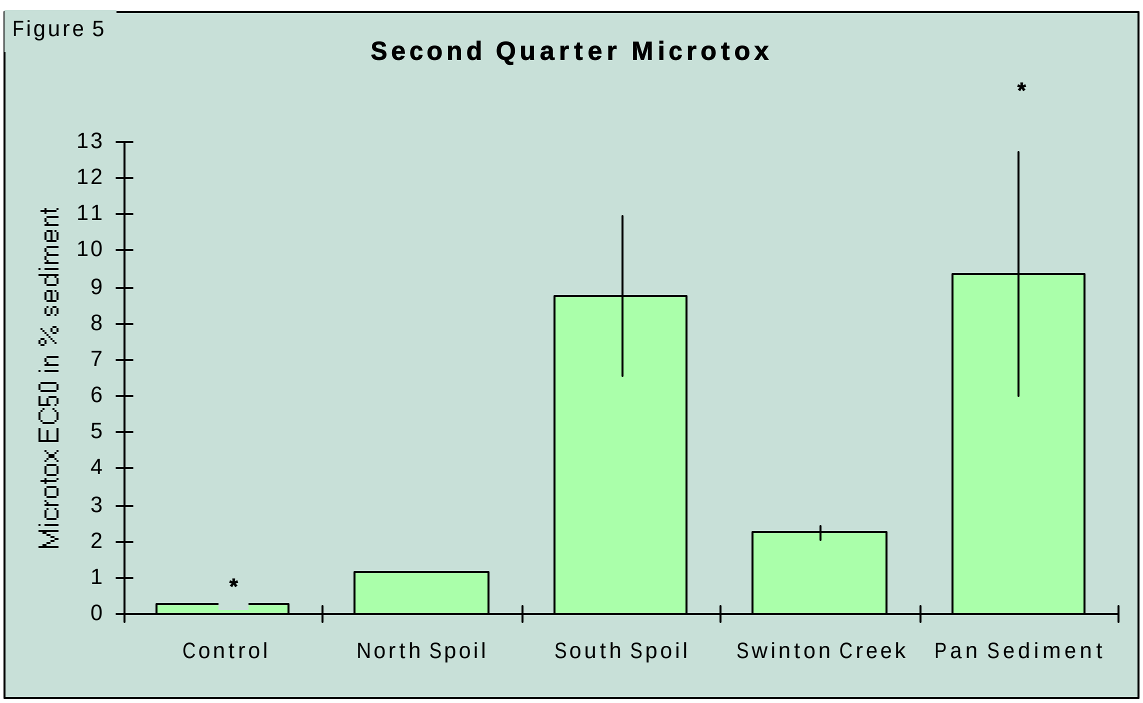


Figure 6

Third Quarter Microtox

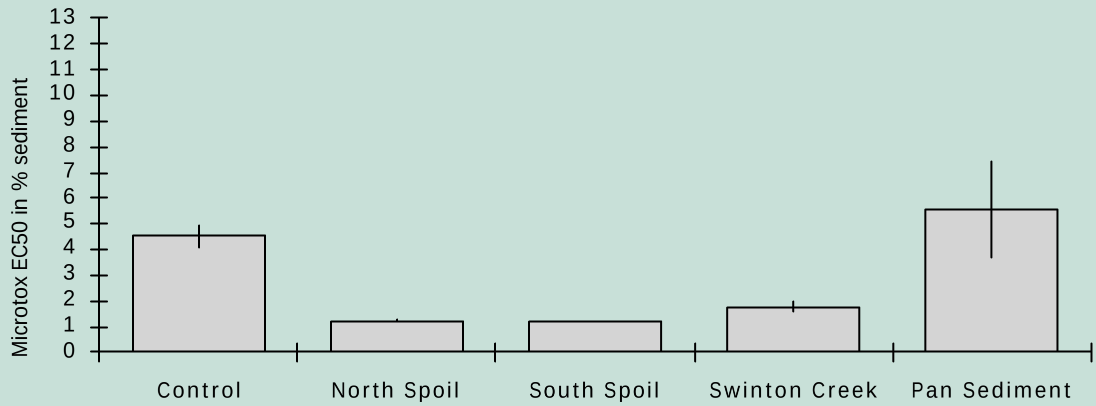


Figure 7

Fourth Quarter Microtox

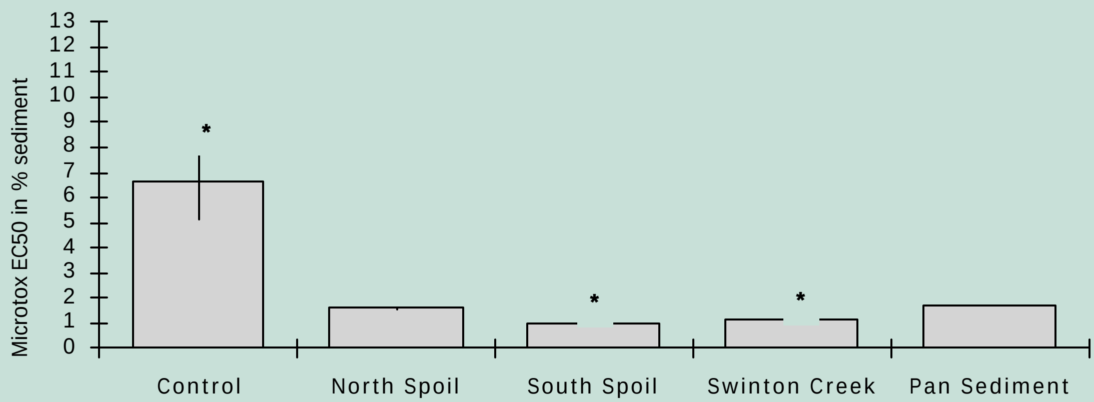


Figure 8

Yearly Averages for 1994

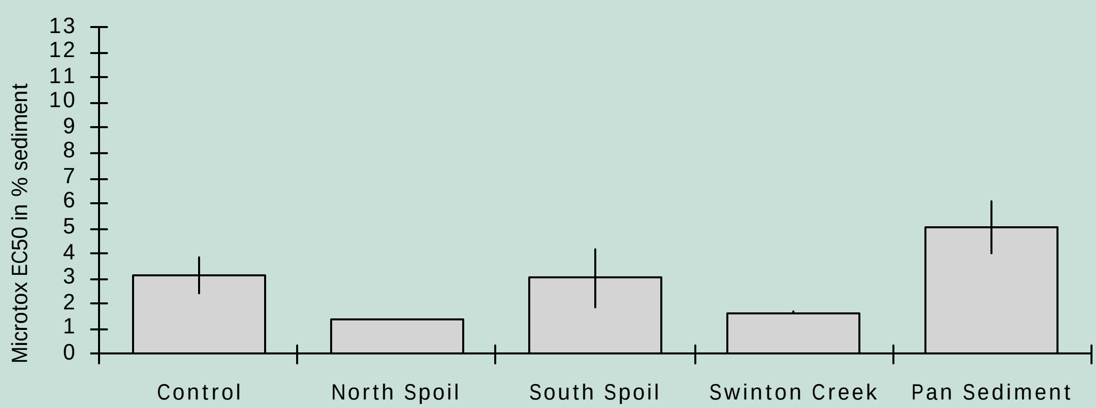
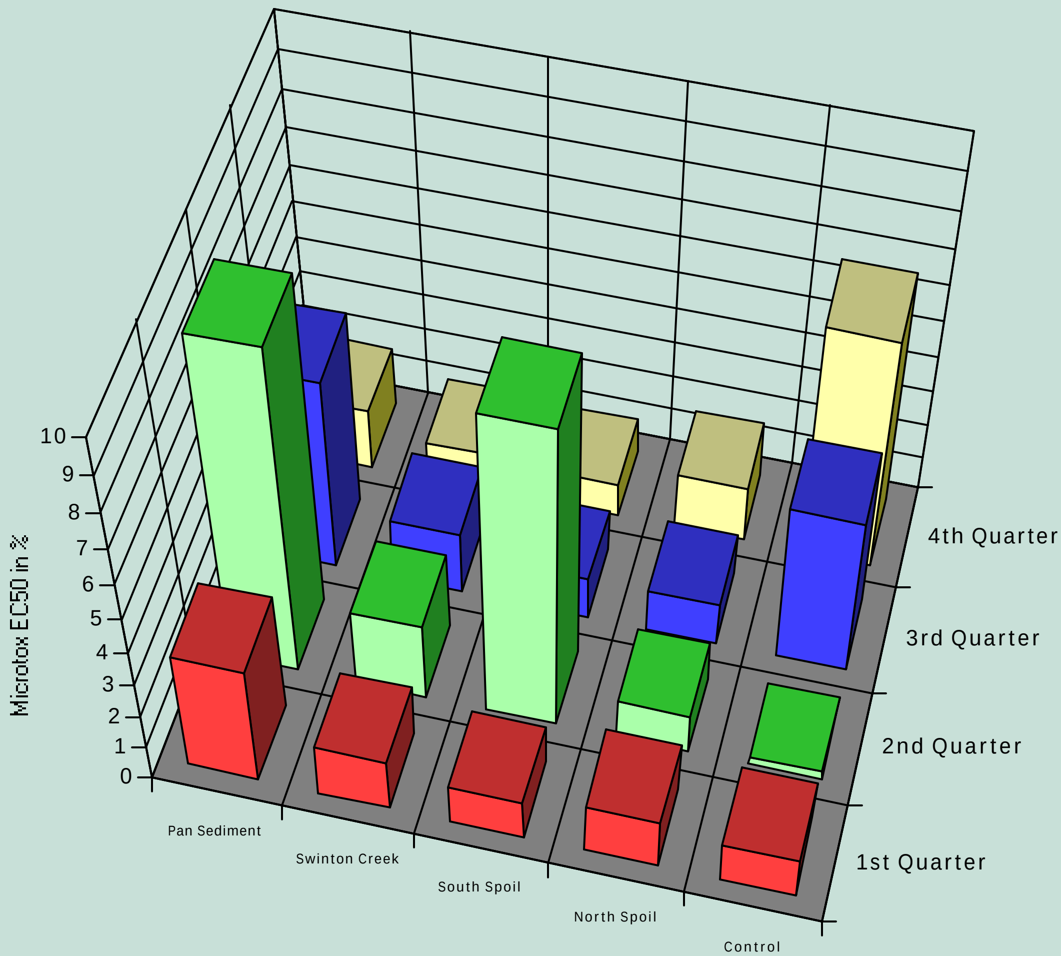


Figure 9



Microtox Averages for 1994

Table Four. Microtox data

First Quarter — Microtox EC50s in percent sediment

	Control	North Spoil	South Spoil	Swinton Creek	Pan Sediment
Significant					
Average	1.1499	1.4559	1.1525	1.5128	3.4728
Standard Deviation	0.1350	0.2345	1.2071	0.3202	1.8080

Second Quarter — Microtox EC50s in percent sediment

	Control	North Spoil	South Spoil	Swinton Creek	Pan Sediment
Significant	X				X
Average	0.2597	1.1427	8.7622	2.2338	9.3759
Standard Deviation	0.0478	0.0879	2.5743	0.7275	3.1768

Third Quarter — Microtox EC50s in percent sediment

	Control	North Spoil	South Spoil	Swinton Creek	Pan Sediment
Significant					
Average	4.5361	1.2338	1.2164	1.7843	5.5452
Standard Deviation	1.1403	0.4369	0.0446	0.7634	2.3710

Fourth Quarter — Microtox EC50s in percent sediment

	Control	North Spoil	South Spoil	Swinton Creek	Pan Sediment
Significant	X		X	X	
Average	6.6429	1.5999	0.9796	1.1176	1.7133
Standard Deviation	2.1442	0.3173	0.5401	0.3689	0.2941

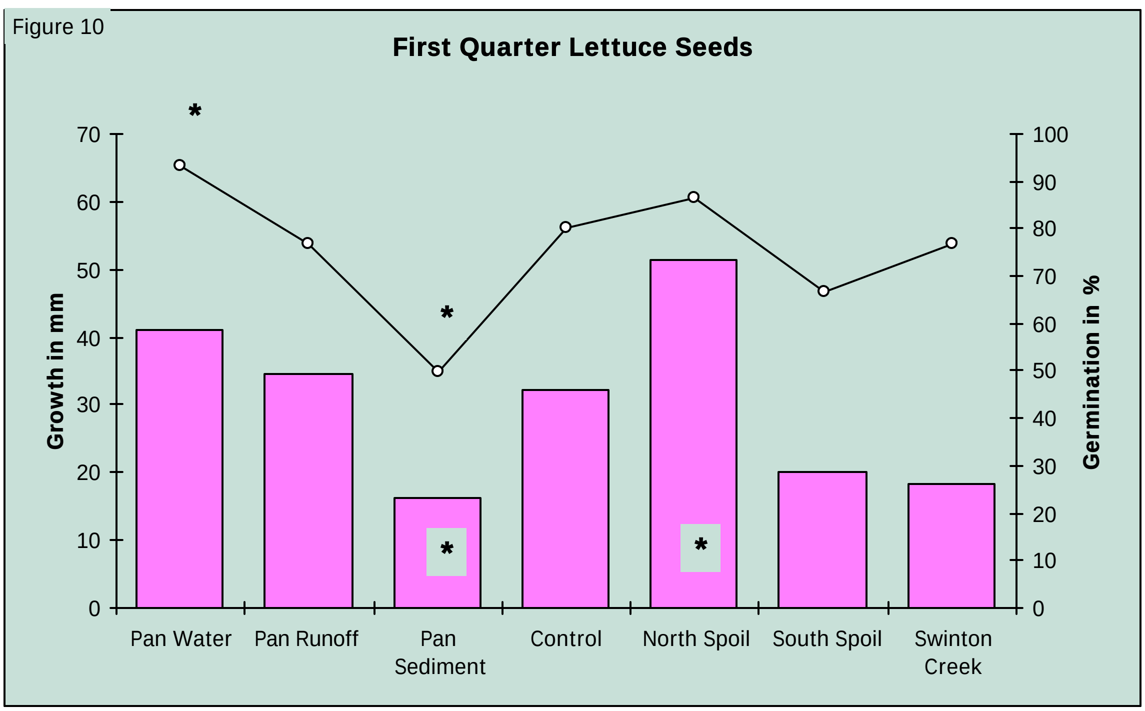
Yearly Averages — Microtox EC50s in percent sediment

	Control	North Spoil	South Spoil	Swinton Creek	Pan Sediment
Significant	X		X	X	
Average	3.1472	1.3581	3.0277	1.6621	5.0268
Standard Deviation	2.9707	0.2081	3.8243	0.4692	3.2953

Lettuce

The lettuce quarterly and yearly results for growth in millimeters and germination in percent are expressed in figures 10 through 16 and table 5. The variance prohibited use of parametric statistics, so the points that are marked with asterisks as statistically different were generated with a Kruskal-Wallis test and nonparametric multiple comparisons. The power of the yearly comparison for growth is 40% so there is a 60% chance that this is a false negative. The power of the yearly comparison for germination is less than 30% so there is a greater than 70% chance that this is a false negative.

One noticeable but not statistically significant trend is that the sediment samples that theoretically had the highest amounts of bridge runoff (the spoil areas) generally had the most growth and germination of all of the sediment samples.



Figures 10-16
These are graphs of the lettuce germination and growth data from 1994. The bars represent growth in millimeters and the line indicates the percent germination averaged from three replicates. The asterisks indicate statistically significant differences from a Kruskal-Wallis nonparametric ANOVA and nonparametric multiple comparisons. Alpha is 0.05.

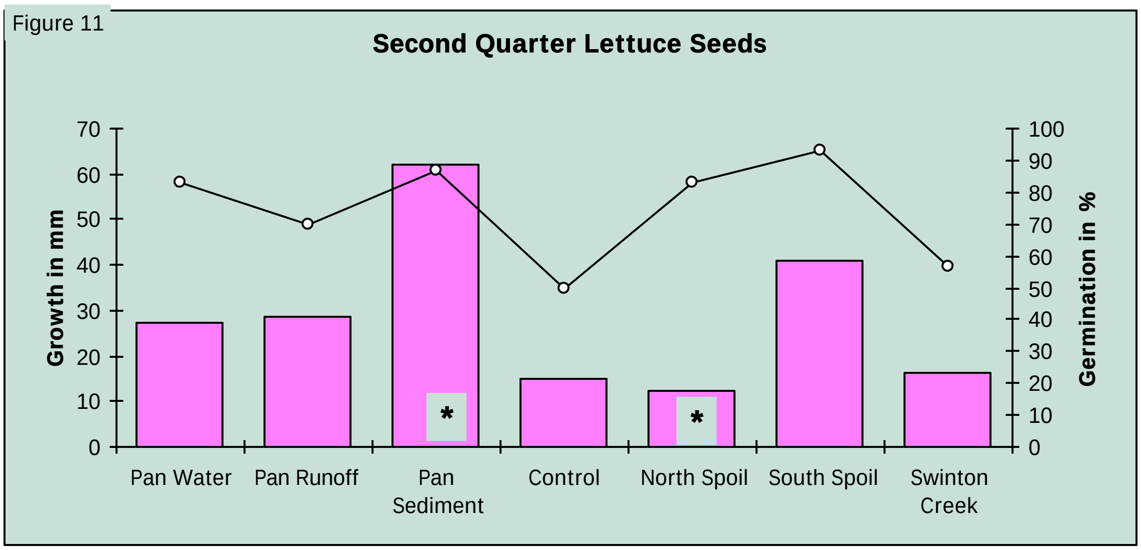


Figure 11
Second Quarter Lettuce Seeds
Growth in mm
Germination in %
70
60
50
40
30
20
10
0
100
90
80
70
60
50
40
30
20
10
0
*
*
Pan Water
Pan Runoff
Pan Sediment
Control
North Spoil
South Spoil
Swinton Creek

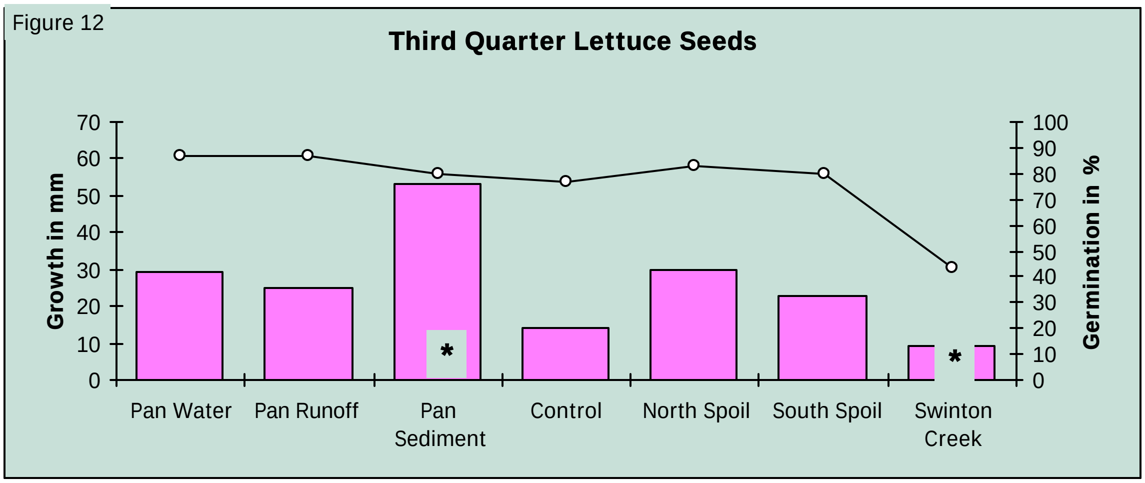


Figure 12
Third Quarter Lettuce Seeds
Growth in mm
Germination in %
70
60
50
40
30
20
10
0
100
90
80
70
60
50
40
30
20
10
0
*
*
Pan Water
Pan Runoff
Pan Sediment
Control
North Spoil
South Spoil
Swinton Creek

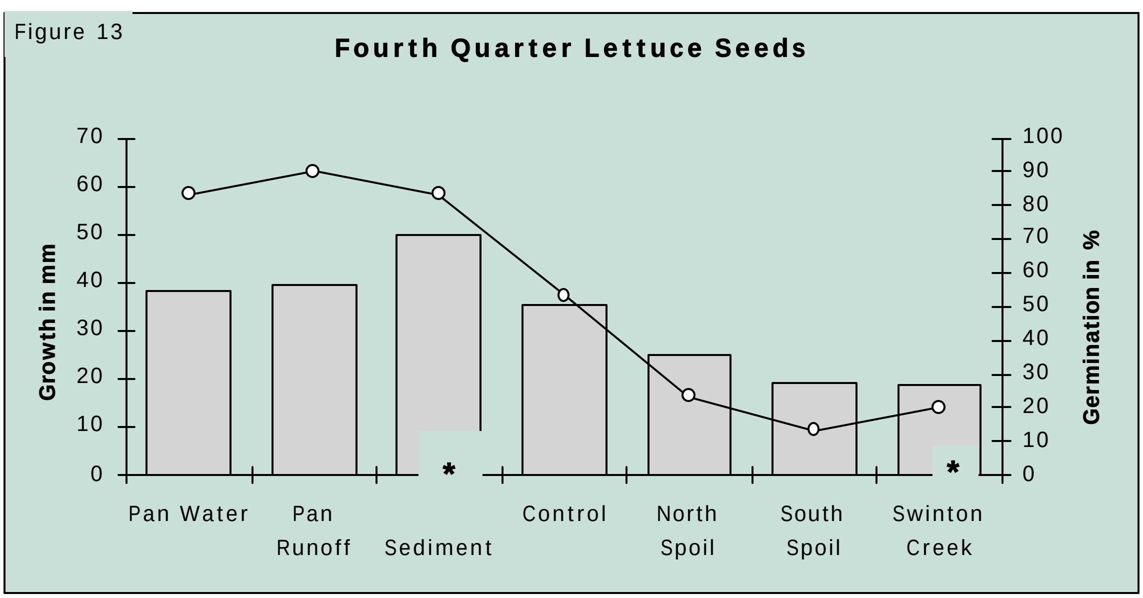


Figure 13
Fourth Quarter Lettuce Seeds
Growth in mm
Germination in %
70
60
50
40
30
20
10
0
100
90
80
70
60
50
40
30
20
10
0
*
*
Pan Water
Pan Runoff
Sediment
Control
North Spoil
South Spoil
Swinton Creek

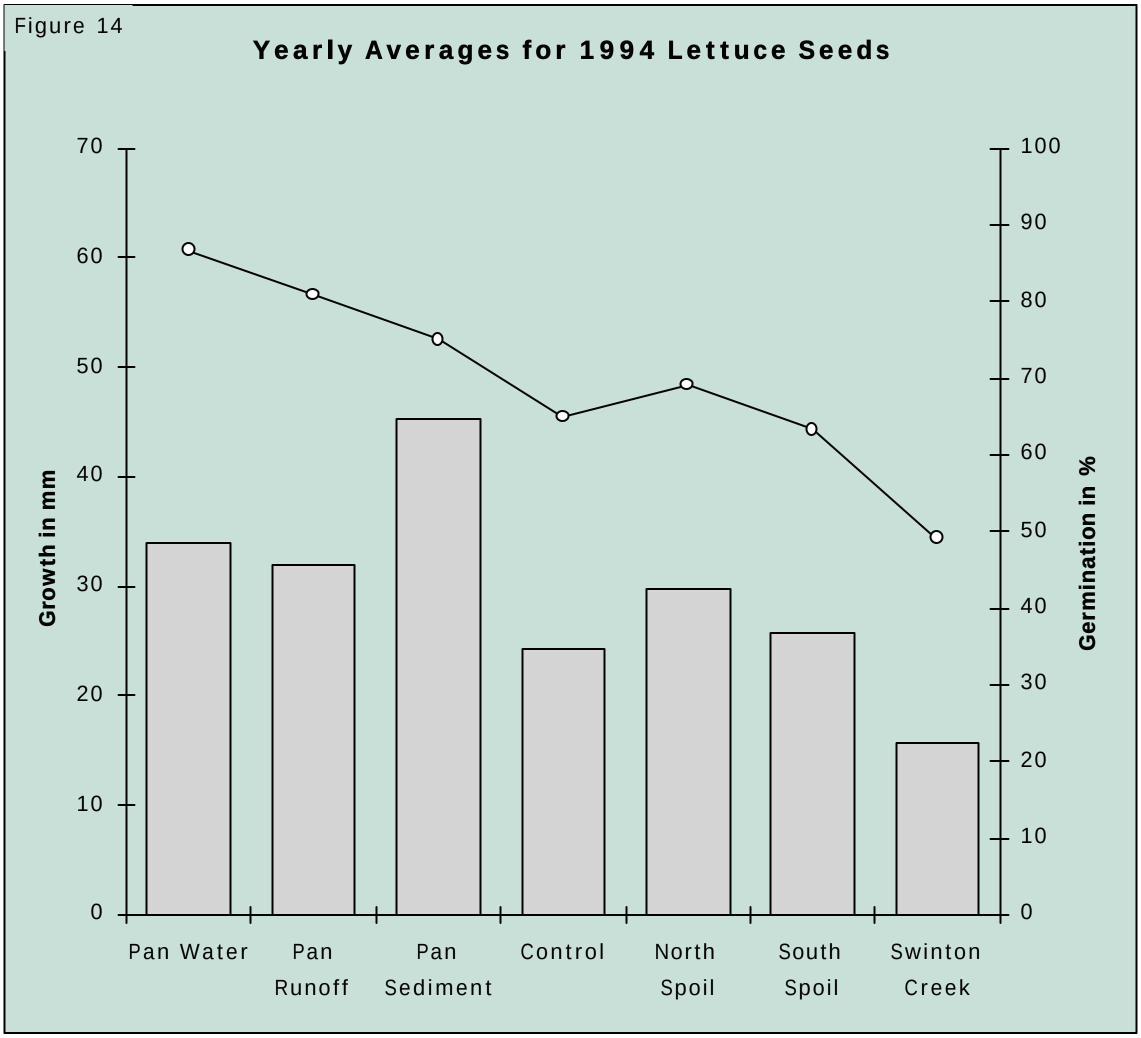
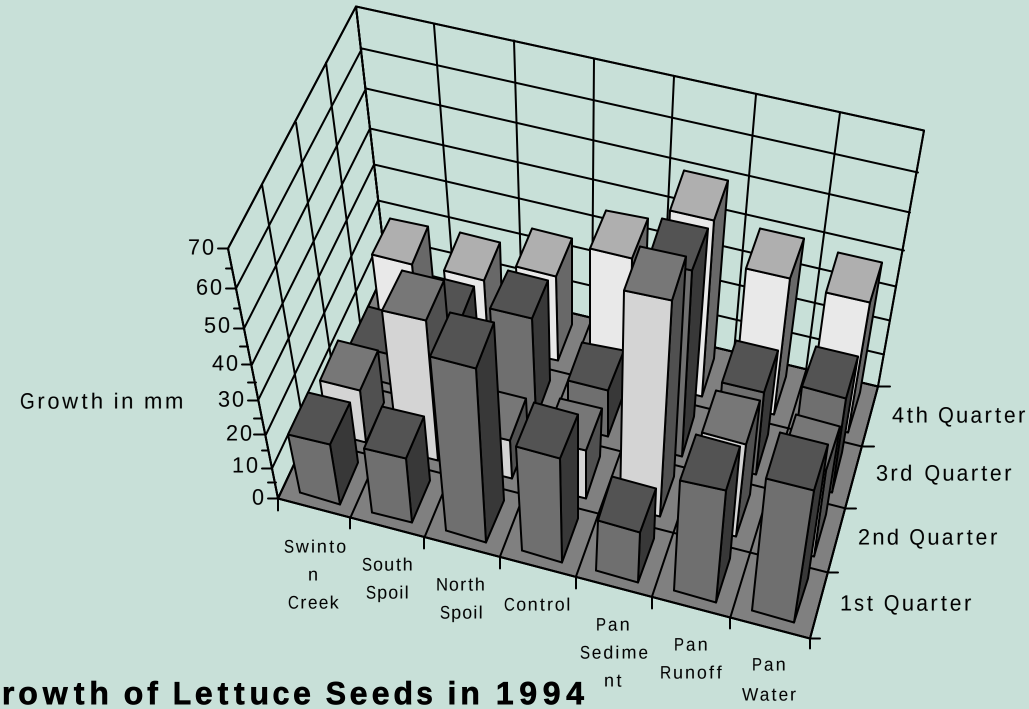


Figure 14
Yearly Averages for 1994 Lettuce Seeds
Growth in mm
Germination in %
70
60
50
40
30
20
10
0
100
90
80
70
60
50
40
30
20
10
0
Pan Water
Pan Runoff
Pan Sediment
Control
North Spoil
South Spoil
Swinton Creek

Figure 15



Growth of Lettuce Seeds in 1994

Figure 16

Germination of Lettuce Seeds in 1994

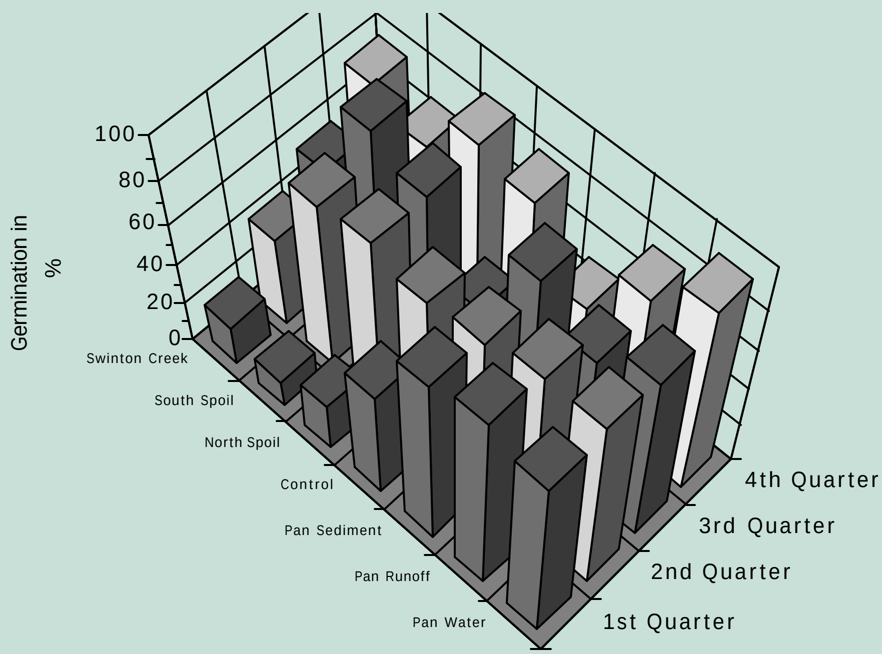


Table 5. Lettuce Data

Lettuce Growth

	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
First Quarter						
Significantly Different			X		X	
	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Average	40.91	34.61	16.21	32.17	51.52	20.00
Standard Deviation	8.62	10.35	2.28	1.45	3.06	5.45
Second Quarter						
Significantly Different			X		X	
	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Average	27.16	28.71	61.91	15.01	12.43	41.13
Standard Deviation	8.40	2.53	2.44	3.42	0.81	6.66
Third Quarter						
Significantly Different			X			
	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Average	29.11	25.13	53.08	14.32	29.85	22.83
Standard Deviation	2.24	2.29	2.37	3.95	7.49	6.69
Fourth Quarter						
Significantly Different			X			
	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Average	38.38	39.68	50.07	35.36	25.17	19.22
Standard Deviation	6.55	5.07	2.44	12.01	8.58	16.65
Yearly Average						
Significantly Different						
	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Average	33.89	32.03	45.32	24.21	29.74	25.80
Standard Deviation	6.77	6.42	20.04	11.11	16.28	10.34

Lettuce Germination

First Quarter

	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Significantly Different	**X**		**X**			
Average	93.33	76.67	50.00	80.00	86.67	66.67
Standard Deviation	5.77	5.77	10.00	0.00	5.77	32.15

Second Quarter

	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Significantly Different						
Average	83.33	70.00	86.67	50.00	83.33	93.33
Standard Deviation	11.55	0.00	5.77	26.46	11.55	5.77

Third Quarter

	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Significantly Different						
Average	86.67	86.67	80.00	76.67	83.33	80.00
Standard Deviation	15.28	15.28	10.00	15.28	15.28	0.00

Fourth Quarter

	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Significantly Different						
Average	83.33	90.00	83.33	53.33	23.33	13.33
Standard Deviation	5.77	10.00	11.55	25.17	15.28	15.28

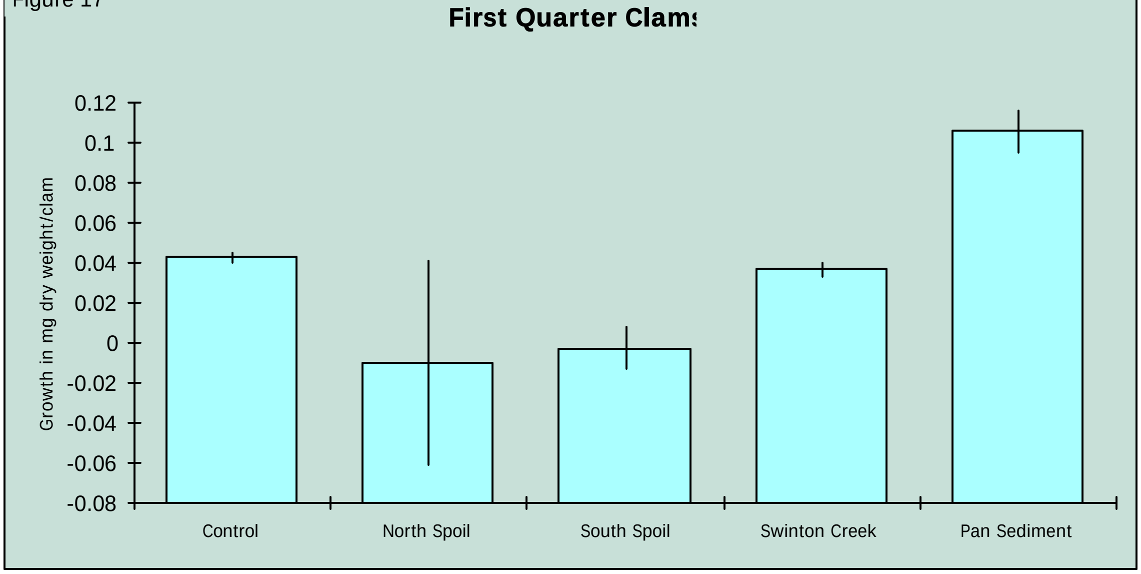
Yearly Average

Significantly Different	Pan Water	Pan Runoff	Pan Sediment	Control	North Spoil	South Spoil
Average	86.67	80.83	75.00	65.00	69.17	63.33
Standard Deviation	4.71	9.18	16.89	15.52	30.60	35.07

Clams

The clam quarterly and yearly growth in milligrams of dry weight per clam results are expressed in figures 17 through 22 and table 6. The variance prohibited use of parametric statistics, so the points that are marked with asterisks as statistically different were generated with a Kruskal-Wallis test and nonparametric multiple comparisons.

Figure 17



Figures 17-22
These are graphs of the clam data from 1994. Each bar is an average growth compared to time zero measured in milligrams dry weight per clam of three replicates. The bars show plus and minus one standard error.The asterisks indicate statistically significant differences from a Kruskal-Wallis nonparametric ANOVA and nonparametric multiple comparisons. Alpha is 0.05.

The comparison data between the four quarters are expressed as a percent of control because each group of clams acquired from the clam farm is from one particular set of brood stock. This means that the clams in one set are very genetically similar, which means the comparisons within one test are very good. This also means that two sets of clams gotten from the Atlantic Littleneck Clam Farm at different times could be fairly genetically dissimilar. This makes comparisons between tests weaker. The yearly comparisons are expressed as a percent of control to overcome these problems.

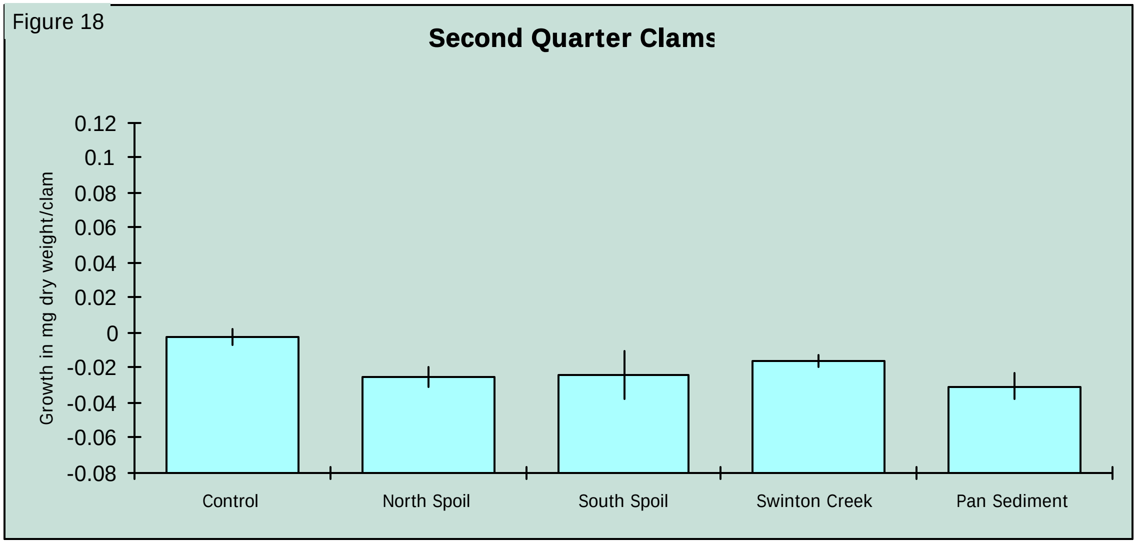
Table 6. Clam Data

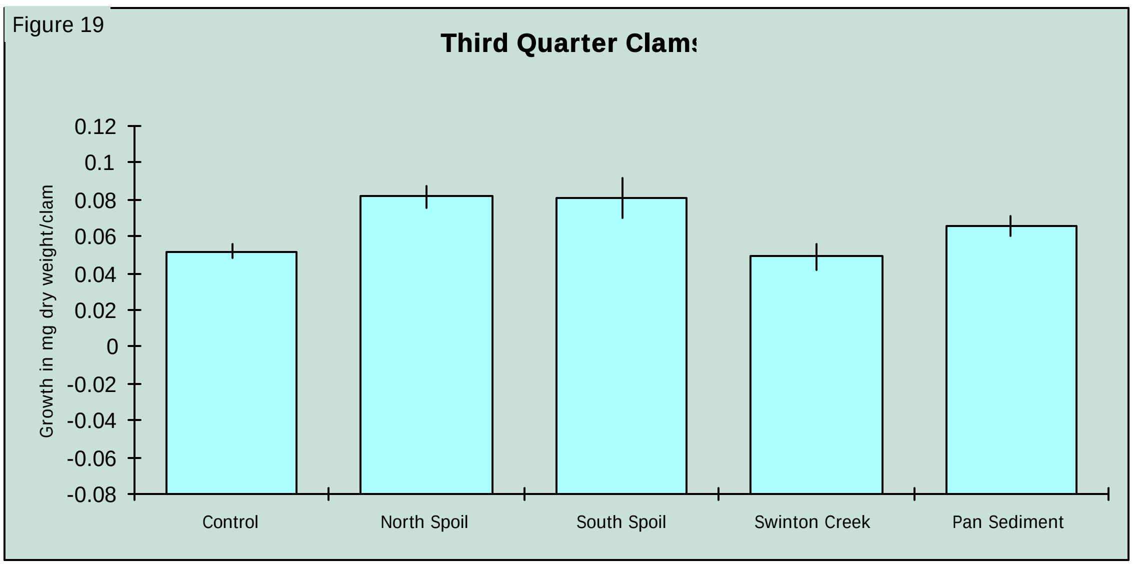
Growth in mg dry wt / clam

	Control	North Spoil	South Spoil	Swinton Creek	Pan Sediment
First Quarter					
Average	0.0425	-0.0100	-0.0026	0.0368	0.1056
Standard Deviation	0.0045	0.0886	0.0184	0.0060	0.0181
Second Quarter					
Average	-0.0019	-0.0251	-0.0241	-0.0157	-0.0303
Standard Deviation	0.0078	0.0106	0.0229	0.0059	0.0120
Third Quarter					
Average	0.0520	0.0815	0.0809	0.0490	0.0659
Standard Deviation	0.0063	0.0102	0.0192	0.0122	0.0099
Fourth Quarter					
Average	0.0411	0.0366	0.0242	0.0080	0.0304
Standard Deviation	0.0488	0.0180	0.0460	0.0217	0.1139
Yearly Average					
Average	0.0335	0.0208	0.0196	0.0195	0.0429
Standard Deviation	0.0240	0.0483	0.0454	0.0291	0.0577

% Control

	North Spoil	South Spoil	Swinton Creek	Pan Sediment
First Quarter	73%	77%	97%	132%
Second Quarter	89%	89%	93%	86%
Third Quarter	124%	124%	98%	111%
Fourth Quarter	98%	98%	85%	95%





The first and second quarter clam growth assays were not run until August, so the sediments were very old. It took several tries to make the assay workable. The older sediments either caused the clams to decrease in weight compared to the time zero clams or they grew very little. This means that the variance in the change in weight was very high when the first two quarters are compared to the second two quarters. The power of the yearly comparison is less than 30% so there is a greater than 70% chance that this is a false negative.

Figure 20

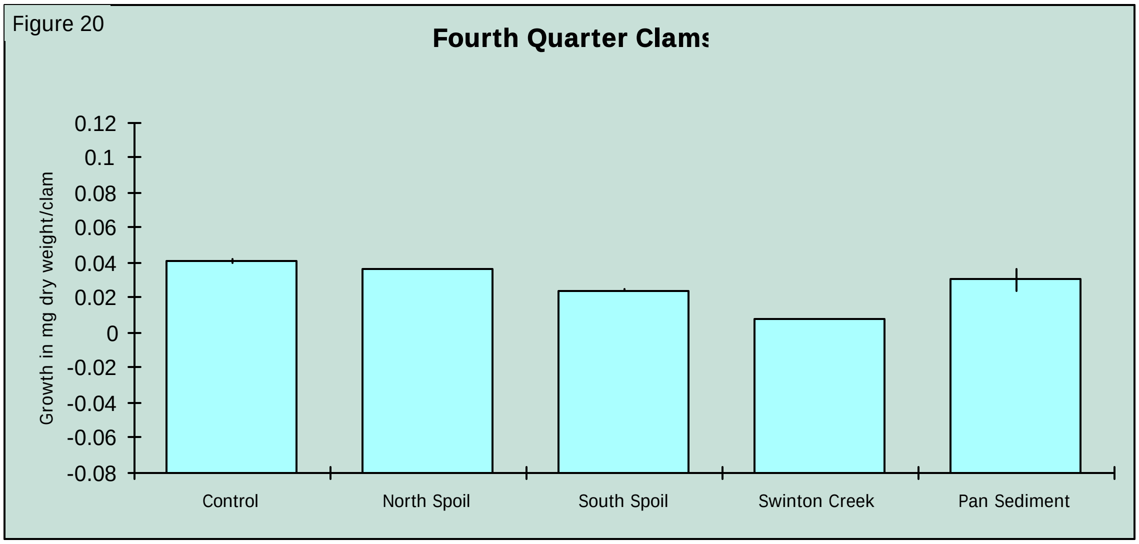


Figure 21

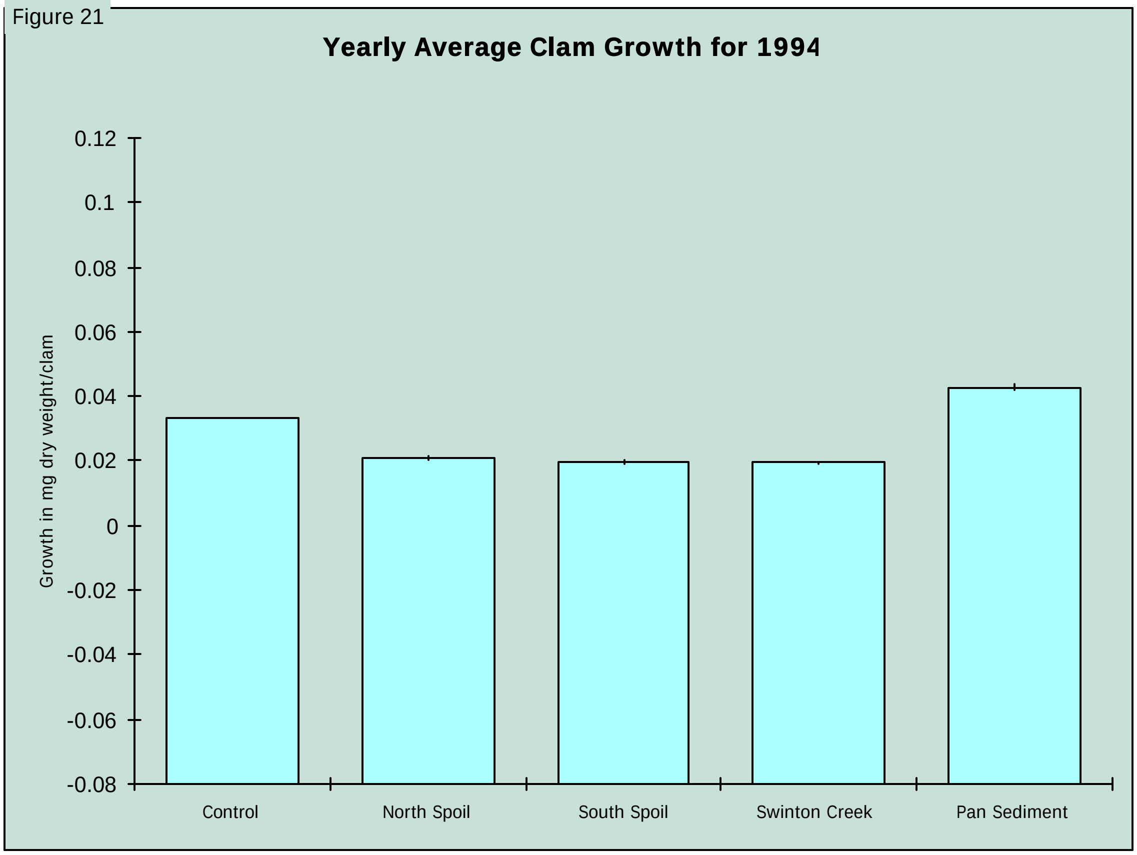
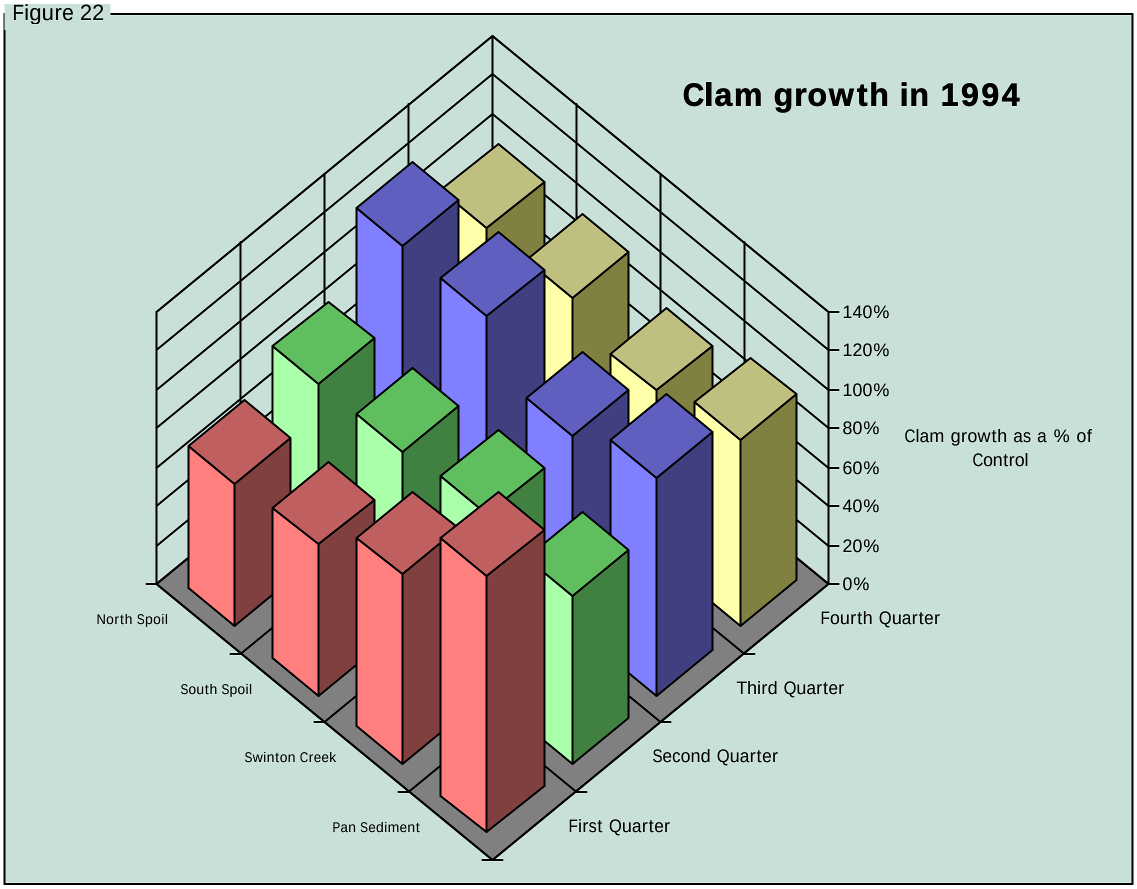


Figure 22



V. Conclusions

Background

The main factors that affect the extent of the impacts of highway runoff (in approximate order of importance) are: the traffic characteristics, the highway design, the surrounding land use, the climate and accidental spills (FHWA 1985). The predicted ADT on the IOP connector for the year 2000 is 8,500 VPD, and in 2005 it is 12,900 VPD (SCDOT 1979,1986) Currently, the estimated traffic on the bridge is no more than 7,000 VPD (Harry Mills, personal communication). This is a very small amount of traffic compared to most sites that are studied for the effects of highway runoff.

From the literature, it appears that sites that have this small of an amount of traffic probably will not show large biological effects. Metals concentrations in a Virginia study showed that lead, nickel and zinc concentrations were highly correlated with traffic volume (Van Hassel *et al*. 1979). This same study showed that a site that had an average daily traffic of 6,550 vehicles per day showed absolutely no statistical differences in metal concentrations from a control site. In a 1985 FHWA study that used fathead minnow (*Pimphales promelas*), the cladoceran, *Daphnia*, and the isopod, *Asellus* showed absolutely no lethal effects from highway runoff from highways with ADT of 7,400 and 120,000 VPD. The amphipod, *Gammarus*, when exposed to runoff from a highway with an ADT of 120,000 showed less mortality than the controls. The mayfly *Hexagenia* was very slightly sensitive in a growth/hatching study, with more than a 20% inhibition of hatching. The alga *Selenastrum* showed some inhibition of growth when exposed to runoff from a site that had an ADT of 7,400 VPD when they were exposed to pure, undiluted runoff (FHWA 1985). The University of Washington performed a study on alga exposed to runoff from roads with 50,000, 42,000 and 7,700 ADT. The alga showed inhibitory effects only from the 50,000 ADT site. Assays with rainbow trout (*Salmo gairdeneri*) showed no effect with filtered runoff, but exhibited mortality in the two high traffic sites when exposed to pure undiluted runoff. The authors posit that the fish were stressed by the suspended particulates, which caused rapid ventilation and a lowering of gill pH. This allowed the metals to be more easily absorbed by the gills (Portele 1982).

The highway design of the IOP connector is the main factor that makes environmental contamination unlikely. In the literature, it shows that highways that allow the particulates to settle out produce much less contaminated runoff. Bioassays on runoff that had flowed through a "grassy-ditch" type gutter, or had been filtered, showed that these procedures significantly reduced the toxicity of the runoff (Portele 1982). This is further supported by Yousef (1982), who proved that sediments under drain and gutter type bridges had lower metal concentrations than sediments under bridges with scupper drains. The pans on the IOP connector seem to trap the particulate phase of the runoff, and like the highways in these examples, this lessens the toxicity of the runoff that actually goes into the marsh. The spoil areas allow for sedimentation which should lessen impacts in the area.

The climate and geography of the IOP connector seem to also make the possibility of contamination low. The tidal flux in this area is great enough to rapidly dilute out many toxins to below detectable limits, or carry them out to the intracoastal waterway. The catch-pans could be a major advantage in case of an accidental spill. If the pans were not full, they would catch the spilled materials, and hold them until a clean up team arrives.

Conclusions from the Bioassays

It appears that in the first year of monitoring, that there were no significant trends in toxicity in any of the sites from any of the bioassays. The effects of highway runoff are historically very subtle at low traffic volumes. It would have been unusual to see high toxicity from these sites even if the connector had been a conventional bridge because the ADT is so low.

The Microtox data hold no statistically significant trends. Generally the pan sediment actually shows a less toxic EC_{50} than any of the other sites. This could be due to nitrogen and phosphorus enrichment in the pans. The fact that the pan water and pan runoff samples seem to stimulate the Microtox also tend to support this.

Lettuce germination and growth show no statistically significant trends, but are generally elevated in the pan water and pan runoff samples. This could be a stimulatory effect (or hormesis) due to small concentrations of contaminants. Simulation might be due to the fact that these samples are *freshwater* samples and the other samples are *estuarine* samples. The salt would definitely inhibit the growth and germination of the seeds. There is a trend toward hormesis in the pan sediment samples. Even small quantities of highly toxic metals like lead and mercury have been shown to cause stimulatory effects in plants (Mrozek 1980,1982). A 1980 California department of highways study showed that the effect of runoff on an alga could be stimulatory or inhibitory depending on the concentrations of heavy metals and nutrients in the runoff. It also showed that in some areas that had low ADT, the runoff was generally stimulatory except after a long dry period. After a dry period there was an inhibitory, and then a stimulatory phase. (Winters 1980). This provides a possible explanation for the increase in growth and germination in the spoil areas compared to Swinton Creek.

The clam growth data are somewhat confusing because of the negative change in weight in some of the first and second quarter samples. There is not a statistically significant trend, but the pattern of growth is roughly similar from all four quarters. The pan sediment may have a slight positive effect on the growth of clams.

Variance within the system

The variance inherent in this system must be taken into account in the conclusions from these data. The salt marsh is a highly dynamic system with tides constantly moving the sediments around. For instance, the spring and winter spoil area samples had a much higher sand content than the summer and fall spoil area samples. The change in grain size from quarter to quarter could have affected bioassay results irrespective of the presence or absence of toxic contaminants. Generally the variance in response in one site (for instance from the pan sediment Microtox data) is as large as the variance between the sites. Statistically, this makes it difficult to reject the null hypothesis that these sediments are alike. One important consideration to make is that *power of these tests was low* and therefore *the chance that this is a false negative is high*.

Lack of chemistry

Originally, it was planned to take sediment and water samples for chemistry to assess the metal and polycyclic aromatic hydrocarbon (PAH) contamination of the sites. There are about ten metals and about twenty organic chemicals that are historically the principal contaminants of highway runoff from concrete surfaces. The chemistry from these samples would have supported the results of the bioassays, and would have added real insight to any regulatory decisions made about the Isle of Palms connector. The chemistry would have allowed management to compare the contamination (or lack thereof) to federal and state legislation, and take appropriate action. The chemistry is the main legal tool for a regulatory agency. The bioassays can measure the combined effects of the contaminants, but will not provide much in the way of a legal basis for regulation.

If the Department of Transportation eventually provides funding for this survey, it will be possible to perform chemical analyses on archived samples. Chemistry data would certainly help in the interpretation of bioassay results.

VI. Recommendations

Due to the low statistical power, it is critical that this system be monitored for a longer time period than one year. Large variance and small sample size contribute to low power. If this study was refined to reduce the variance, and continued for a few more seasons to increase the sample size, then the power of the statistical analysis would increase and one of two things would happen. 1) If there is truly no statistical difference in the sites, a valid conclusion of no difference could be reached (i.e. the null hypothesis will be accepted with high statistical power). 2) If there is a difference in the sites, a larger sample size will enable the study to discover it.

At this time, no statistically valid policy recommendation can be made except to encourage further study of the system. It is crucial that the study be continued because the current statistically insignificant trends could change to significant ones as the average daily traffic increases.

Refinements to the study

There are several ways to change this study that would make it more capable of assessing whether the contaminants from the bridge are being accumulated in the sediments under the pans and in the spoil area in biologically significant concentrations.

1) The sampling sites should be changed.

The Pan Runoff sample should be either standardized so that it will be constant from quarter to quarter, or dropped. Sometimes the runoff was collected from the beginning of a rainstorm when the load of contaminants was theoretically the highest and sometimes it was collected after it had been raining for two days. The number of dry days before the sample is taken could affect contaminant concentrations (Portele 1982). If there had been a long time since the last rain then there would be a higher pollutant load. If it were possible to get a sample of runoff at the start of a rainstorm after seven consecutive dry days every quarter then the data would be standardized enough to make rigorous comparisons. It might be more feasible to sample the runoff as it comes out of the gutter, before it ever gets to the pans. This might provide a more detailed picture of the system because this should be the most contaminated that the runoff ever gets: it is fresh from the highway, and the particulates have not had time to settle out.

A direct sample of rain water should be taken every quarter to see if the stimulatory effects in the pan water are coming from materials from the bridge or materials already present in the rain. The sample from Swinton Creek (if possible) should be collected by hand, the same way that the other sediment samples are collected. Using a sediment grab disturbs the layers of sediment so that it is impossible to collect just the upper oxidized layer. This probably attributes to the variance in the Swinton Creek samples from quarter to quarter. This kind of sampling can be done by jon-boat or any boat with a shallow enough draft to get into Swinton Creek at low tide.

Only one spoil area sample is really necessary. The north and south sides of the spoil area differ physically because the north side is higher in sand content and lower in silt/clay content than the south side. The spoil area sample should be taken from whatever area has sediment characteristics similar to Swinton Creek.

It is possible that the Wild Dunes/Dewee's Inlet is affected by urban runoff from the development in Wild Dunes, or runoff from the extensive golf courses that could contain fertilizer or pesticides. That particular area has a slightly different range of natural influences than Swinton creek (tides, currents, rainfall, vegetation) in addition to different types of anthropogenic influences. A better "clean sediment" control would be from another part of Swinton Creek that is not affected by the bridge runoff. In a study in Tacoma, Washington, sediment chemistry showed that levels of zinc, lead, chromium and cadmium from a roadway with an ADT of 80,000 faded to background levels 400 meters away from the roadway (Wisseman and Cook 1977). In the Isle of Palm Connector Study, a sample from a creek bank several hundred meters from the bridge might better illustrate what clean IOP watershed sediment should be like.

2) The number of replicates of the Microtox should be changed.

The Microtox assay is the most expensive assay to run. Doing samples in duplicate instead of triplicate should be sufficient. This might allow more replicates to be run with the lettuce and clam tests.

3) The average daily traffic of the bridge should be estimated on a quarterly basis.

This would enable administrators to ascertain roughly how much contamination should be expected from the bridge. The amount of traffic is higher in the summer because many people use the bridge to get to the beach. The ADT can be taken as a covariate in the statistical analysis which will reduce the variance.

4) Sample porewater salinity should be measured.

This will enable the researcher to account for salinity, so that it can be factored out of the statistical analysis.

5) Replace lettuce with a more ecologically relevant organism.

Some preliminary studies were done to assess the worth of *Spartina alterniflora* in a germination study instead of lettuce. On the East coast, *Spartina alterniflora*, the smooth marshgrass, is the base of the food web of the salt marshes and estuaries (Teal 1962). The plant materials are broken down by the mechanical action of tides, waves, currents and storms and by microbial action, and then the detritus is eaten by many animals (Teal 1962, Odum and de la Cruz 1963, 1967, Darnell 1967a, 1967b). Because it is the base of the Isle of Palms watershed ecosystem, *Spartina alterniflora* is a keystone species, a species "in whose absence dramatic alterations to the ecosystem would occur," (Paine 1980).

Mature stands of *Spartina alterniflora* are generally pollution resistant and can be found growing in even the most polluted estuaries on the East coast. The seeds are probably also resistant enough not to die in most toxicity testing situations, but the percent germination and the growth of the seeds can be very sensitive to many types of toxicants like hydrocarbons and metals (Hershner and Lake 1980, Mrozek 1980, 1982).

Several authors have published methods for growing *Spartina* seeds to determine the impacts of various factors on germination and growth (Mooring *et al*. 1971, Mrozek 1980, Mrozek 1982, Seneca and Blum 1984, Waddel and Kraus 1990, Walsh 1990). The American Society for Testing of Materials (ASTM) has recently developed a proposed root elongation assay with several species of aquatic plants, including *Spartina alterniflora*. These sources do not agree on a standard procedure, and are often contradictory.

Most of the authors mentioned above agree on the following steps: 1) Seeds can be ordered from February to September from Environmental Concern Incorporated ((410)-745-9620 P.O. Box P, St. Michaels MD 21663), a company that harvests seed from North Carolina marshes. Environmental Concern stores the seeds in 4% seawater at 4°C, which maintains the germination in the best of conditions to around 50% (Mooring *et al*. 1971). 2) They should be sorted by size, so that all seeds used are from the same size class, and damaged seeds should be discarded. 3) They should be rinsed in a bleach solution for a few minutes to kill fungi and then rinsed with deionized water to remove the bleach. 4) Then test material and water are added to a culture chamber and then seeds are added. 5) Then they are incubated. Unfortunately, the literature is often contradictory on the optimal parameters for these steps.

There are several important questions about this procedure that have not yet been answered. One question is how long are the dry seeds ordered from Environmental Concern going to be viable? What is the optimal way to store the seeds once they have been received, but before they are tested? No one has experimented with how much water or sediment test solution is appropriate, but Mooring *et al*. (1971) determined that using fresh water increases the percent germination. Many seed bioassays are run in a petri-dish with filter paper on top of a solid test substance or in a liquid test substance. There are no recommendations about what kind of filter paper, if any, seems to work best, or if a plastic seed tray is more appropriate than filter paper. The most suitable number of seeds in a culture chamber is unknown, as is what kind of culture chamber works best. Table 7 summarizes several authors' methods for germinating *Spartina alterniflora*.

Table 7 is a summary of several authors differing proceedures for germinating *Spartina alterniflora*.

Author	**Light:Dark**	**Thermo-period**	**# Seeds per petri dish**	**Days incubated**	**Test solution**	**Other**
Mooring *et al.* 1971	24 Dark	8h 35°C: 16h 18°C	20	30	3 ml	Dissect out embryo
Mrozek 1980, 1982	24 Dark	8h 35°C: 16h 18°C	50	30	10 ml	Do not dissect out embryo
Seneca and Blum 1984	24 Dark	"35 - 20" Unclear	Not reported	25	"Moist filter paper"	
Walsh 1990	16 : 8	16h 35°C : 8h 20°C	Not reported	Not reported	Not reported	
Waddell and Kraus 1990	12 : 12	27°C	20	18	5ml	
ASTM	16 : 8	8h 35°C : 16h 18°C	10-15	10	5 ml	

After the incubation period, percent germination and root length compared to control are common endpoints measured. Root biomass, shoot length and dry weight are also usable endpoints. This test is much more sensitive than a mortality test. Even if a substance is not toxic enough to kill the seed, it could show subtle effects on the germination or early growth of the plant.

Once these questions are answered and the various problems that are sure to arise with this system are solved, a *Spartina alterniflora* seed germination/root growth assay will be a very useful test for this kind of system. Unfortunately, in the preliminary experiments the germination was very low and declined as the seeds were stored for longer periods of time.

6) The clam assay should be run in artificial seawater in an incubator.

The seawater for the clam assay was from the Grice Marine Biological Laboratory. This seawater varies slightly in salinity and pH throughout the year. The water is collected from Charleston harbor, and contains small amounts of contaminants from the harbor, which vary slightly throughout the year. The assays were performed in the wet lab at Grice, where the temperature varies from near 24 degrees Celsius in the summer to around 16 degrees Celsius in the winter. The clams grow faster in warmer weather. The variation in seawater and temperature contribute to the variance in clam growth from quarter to quarter. Artificial seawater and an incubator (or a room with a more constant temperature) will help reduce the variance.

7) Chemistry should be taken at all sites at least once a year.

This will quantify what contaminants are in the system and in what quantities. After this information is obtained, then the study can be refined again to assess the biological impacts of those specific contaminants.

8) Shift emphasis of study towards detection of eutrophication.

From the literature and research it seems probable that before this system starts to exhibit any toxic or inhibitory effects, it will go through a period where it exhibits stimulatory effects. Assays should be investigated that are sensitive to nutrient enrichment. It might make more sense to replace one or more of the currently used assays with an organism that will exhibit a greater response to eutrophication. Algal assays might be a useful tool to determine the degree of eutrophication.

Directions for future research

One factor that could not be assessed by this study was the rate of accumulation of particulates, debris and trash in the pans. This is an important factor in the maintenance of the bridge, because it will determine how often the pans should be cleaned out. Perhaps one section of the pans (roughly sixty or so) could be set aside in January. Every month, five of the sixty pans could be cleaned out, and the wet weight, wet volume, dry weight and percent water of the pans' contents could be measured. At the end of the year the accumulation rates of sediment in the pans, and the optimal time between cleanings could be calculated. It might be useful to do bioassays or chemical analyses on the contents of these pans to see if there is a change in toxicity of the contents as they age.

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